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MORPHOLOGICAL AND BIOCHEMICAL RESPONSES OF MAIZE (*ZEA MAYS* L.) TO OIL FIELD WASTEWATER POLLUTION

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Abstract

Agricultural sector is challenged with a number of factors including those arising from rapid industrialization, which is the key point responsible for reductions in food production. Industrial waste oil contamination has been determined with negative impacts on plants. Although several studies have demonstrated the impact of crude oil on crops, but the effects of oil field wastewater concentrations on maize growth and biochemical activities are poorly understood. Therefore, the present study investigated the effects of 1, 5 and 10% oil field waste water concentrations on morphological and biochemical growth parameters of maize. Both oil field wastewater and soil samples contained heavy metals including Zn, Pb, Fe, Cu, Cd and Cr. Gradual decrease in seed germination (%) was observed with the increase in oil field wastewater concentrations after 48, 72 and 96 h. Application of oil field wastewater concentrations reduced seedling growth including shoot/root length and number of leaves. Ionic quantification analyses showed that Na^+ and Ca^{+2} ions decreased in shoot while increased in roots. Potassium (K^+) ions increased in shoot while decreased in roots at 5 and 10% oil field wastewater concentration. Photosynthetic pigments (chlorophyll "*a*", "*b*" and carotenoids) and total soluble sugar significantly decreased in maize seedlings as the concentrations of the oil field wastewater increased. Higher concentrations of oil field wastewater significantly increased antioxidant enzymes such as catalase, ascorbate peroxidase, superoxide dismutase and peroxidase enzymes in maize seedlings to cope with the stress conditions. These results suggested that oil field wastewater adversely affects maize morphological and biochemical growth parameters.

Key words: Maize, Oil field, Wastewater pollution, Heavy metals

Introduction

Effects of oil and gas exploration have been widely accepted on all environmental entities in general and on soil and water in specific leading to hazardous consequences on various living organisms. Cumulative oil and gas exploration during their processing activities contaminate the surrounded water that's added to the earth surface and commonly called polluted wastewater (Arthur *et al*., 2005). Plants experiences stunted seedling growth and chlorosis of leaves growing on or near the oil polluted soil (Terek *et al*., 2015). Plant leaves anatomy is also damaged by application of oil polluted wastewater (Omosun *et al*., 2008) and reduced chlorophyll contents (Baruah *et al*., 2014). Oil field wastewater treatments reduced carbohydrates, proteins and other biochemical constituents in maize and cowpea (Adesina & Adelasoye, 2014). Heavy metals are present in higher concentrations in oil field wastewater which synthesized reactive oxygen species (ROS). Such wastewater causes lipid peroxidation and oxidative damages in plants if used for irrigation purposes (Villasante *et al*., 2005; Dietz, 2010).

Plants possess defence system consisting of different antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) against oxidative stresses (Mittler *et al*., 2004). Superoxide dismutase is an important enzyme of plant defence system by converting hydrogen peroxide and reactive superoxide radicals into oxygen (Alscher *et al*., 2002). Oil field wastewater application increased CAT contents whereas decreased SOD and POD contents in maize (Olubodun & Eriyamremu, 2013).

Maize is a vital crop and considered as third most cultivated crop after wheat and rice in the world (Suleiman & Rosentrater, 2015). Maize is grown throughout the world for its high nutritional and consumption values (Golob *et al*., 2004). In Pakistan, maize is commonly grown in southern districts. In the last few years, huge amount of wastewater releases from the newly established gas and oil companies which polluted the nearby crop fields due to poor management. Very few research studies have been conducted to evaluate and describe the consequences of oil polluted waste water on plant growth (Olubodun & Eriyamremu, 2013). Keeping in mind the geographical position of most of the oil and gas processing fields and the area under maize cultivation, the existing research was aimed to investigate the detailed consequences of oil field waste water on morphological and biochemical growth attributes of maize.

Materials and Methods

Seeds and samples collection: Maize (*Zea mays* L. *cv.* Azam) seeds were collected from Barani Research Center, Serai Naurang, district Lakki Marwat, Khyber Pakhtunkhwa, Pakistan**.** Wastewater and soil samples were collected from Magyar Oilage Limited (MOL) Oil and Gas Company, Makori, district Karak, Khyber Pakhtunkhwa, Pakistan. Oil field wastewater was further diluted to 1, 5 and 10% (v/v).

Soil and water analyses: The heavy metals analyses of collected soil and oil field wastewater were determined through atomic absorption spectrophotometer (Gul *et al.*, 2015). Soil samples were oven dried at 60°C, ground in mortar and pestle, sieved to a mesh size of 0.5 mm and wet digested. For analysis of oil field wastewater samples, 40 mL water was mixed with 10 mL HNO_3 and placed on hot plate to reduce volume and dissolved the compound. When the quantity reduced to 15 mL, it has been further diluted with 20 mL distilled water and thus prepared the wastewater samples.

Morphological assays: Experiments were conducted in pots filled with sterilized sand and clay in a ratio of 1:1. Three replicates were used for each treatment with 10 seeds per replicate and pots were arranged in complete randomised design (CRD). Experimental pots were kept in dark rooms for seed germination at temperature 30±2˚C for three days. A seed is considered germinated if the epicotyls has the length of 1 cm. After 10 days of seed germination, shoot/root length, number of leaves and secondary roots were determined.

Biochemical assays: For biochemical analyses, maize roots and leaves treated with 1, 5 and 10% oil field wastewater concentrations were collected from maize plants and conducted the following biochemical analyses;

Ions determination: Presence of different ions (Na^+, K^+ and Ca^{+2}) in maize seedlings were determined by using Awan & Salim (1997) protocol with slight modification. Maize roots and leaves (25 mg) were digested separately in H_2SO_4 and H_2O_2 at a ratio of 2:1 and heated for 15 min. After digestion, each sample was added 20 mL distilled water and shifted to shaker for shaking. When shaking completed, samples were filtered through Whattman No. 42 filter paper and the ions were determined using flame photometer.

Determination of photosynthetic pigments: For determination of photosynthetic pigments, maize leaves sample (25 mg) were taken in falcon tube and added 25 mg magnesium oxide (MgO) to prevent pheophytin formation. Samples were homogenized in shaker at 200 rpm for 2 h at 25°C after adding 5 mL methanol. When the shaking completed, samples were centrifuged at 4000 rpm at 25°C for 10 min. The supernatants of the samples were collected in cuvettes and read against methanol as a blank solvent at wavelengths 666, 653, and 470 nm using UV-Vis spectrophotometer (UV-1602, BMC, Canada). Chlorophyll '*a*', '*b*' and total carotenoids contents were determined using the formula described below (Lichtenthaler & Wellburn, 1983);

Chl '*a*' = $15.65\ A_{666} - 7.340\ A_{653}$

Chl '*b*' = $27.05\ A_{653} - 11.21\ A_{666}$

Total Carotenoids = $(1000\ A_{470} - 2.860\ Chl_a - 129.2\ Chl_b) / 245$

Metabolic studies

Determination of total soluble sugars (TSS): Samples (50 mg) of maize fresh leaves and roots were ground in 3 mL hot 90% ethanol and kept in incubator at 60-70°C for 1 h. After 1 h, reaction mixture volume was increased to 25 mL by adding 90% hot ethanol. Sample contained 0.5 mL reaction mixture, 0.5 mL phenol (5%) and 2.5 mL analytical grade sulphuric acid mixed thoroughly and cooled in air for exothermic reaction. Absorbance was noted at 485 nm and glucose solution curve was used as standard to calculate soluble sugar (Shields & Burnett, 1960). The unit mg g-1 fw-1 was used to express sugar.

Determination of antioxidants enzymes: Various antioxidant enzymes activate were determined in maize seedling treated with 1, 5 and 10% oil field wastewater concentrations. Maize leaves and roots sample (0.5 g) were separately ground in ice-cold 50 mM phosphate buffer (pH 7.8) with mortar and pestle. Then, enzyme extract was centrifuged two times at 4°C for 15 min at 12,000 rpm and different enzymes activities were measured from the supernatant collected.

Kumar & Khan (1982) protocol was used to determined peroxidase (POD) activities in maize seedlings. Guaiacol (3 mL) was used as a substrate and 100 µL enzyme extract, 2.7 mL of 50 mM potassium phosphate buffer (pH 6.1), guaiacol 1.5% (100 µL) and 100 µL of 0.4 % H_2O_2 were present in enzyme mixture. Absorbance for POD was determined at 470 nm using Spectro-photometer and expressed as per g FW.

Dhindsa *et al.*, (1981) protocol was used to measure the SOD activity by inhibition of photochemical reduction of nitro blue tetrazolium (NBT). The 4 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 77.12 mM NBT, 0.1 mM EDTA, 13.37 mM methionine, 10 mL of enzyme extract, and 100 mL of 80.2 mM riboflavin (riboflavin was added last). Wavelength of 560 nm was used to determine photo-reduction of NBT and an inhibition curve was drawn against different volumes of extract. SOD activity was expressed in U/g FW.

For determination of ascorbate peroxidase (APX) activity, reaction mixture of 3 mL was prepared from 2.7 mL of 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbic acid (0.1 mL), 2% H_2O_2 (0.1 mL) and enzyme extract (0.1 mL). Absorbance for APX was determined at 290 nm for 1 min. The oxidizing amount of ascorbate was measured by using extinction coefficient (ε = 2.8 mM-1) following the formula (Nakano & Asada, 1981);

APX (mM/g FW) activity+ (activity*A*V/a)/ (E x W)
Activity = OD value

W = Sample fresh weight
V = Buffer solution volume used in enzyme extraction
A = Amount of enzyme extract used in reaction mixture
E = Activity constant i.e. 2.8 mM/cm

For determination of catalase (CAT) activity, reaction mixture was prepared from 10 mM H_2O_2 (25 uL), enzyme extract (100 uL) and 25 mM potassium phosphate buffer (2.7 mL) (Radwan *et al.*, 2006). The H_2O_2 disappearance was noted at 240 nm (E = 0.036 mM−1 cm−1). The final activity was expressed as mM/g FW.

Statistical analysis

Statistics 9 software was used for statistical analyses of the data. The data was examined through standard deviation (SD) and analysis of variance (ANOVA) with significance level (p<0.05) for all treatments. Different alphabets indicates significant difference between means of control and treatments.

Results

Metal analyses in oil field waste water and polluted soil: Different heavy metals were analysed in the collected polluted oil field waste water and soil samples. Both the wastewater and soil samples contained heavy metals containing Zn, Pb, Fe, Cu, Cd and Cr. Results indicated that Fe is present in higher concentration compared to other heavy metals in oil field polluted wastewater samples. Total concentration of Fe was 366.8 mg/L while the 2nd most abundant heavy metal in oil field polluted wastewater sample was Pb with 104.72 mg/L concentrations. Similarly, Fe is present 377.4 mg/L, the most abundant in oil field waste water polluted soil compared to Cu, Zn, Pb, Cd and Cr respectively (Fig. 1).

Effect of oil field wastewater on germination percentage of maize: Higher concentration of oil field wastewater significantly decreased seed germination as compared to control. Maize seeds were germinated after 24 h in control only while no germination was observed in 1, 5 and 10% oil field wastewater concentrations (Fig. 2). At 48 h, maize seed germination was 70, 36, 20% while at 72 h, maize seed germination was noted 70, 50 and 33% at 1, 5 and 10% oil field wastewater concentrations respectively (Fig. 2). These results indicated that higher concentrations of oil field wastewater decreased seed germination of maize compared to control and lower concentrations.

Effects of oil field wastewater on shoot and root length of maize: Seedling length of maize was also affected by oil field wastewater concentrations. The lower concentrations improved shoot/root length of maize. Results indicated that maize shoot/root length was 21 and 12 cm treated with 1% concentration oil field wastewater respectively while 5 and 10% reduced shoot/root length compared to control and 1% oil field wastewater (Fig. 3A and B).

Effects of oil field wastewater on number of leaves and secondary roots of maize: Results indicated that higher concentrations of oil field wastewater decreased total number of leaves and secondary roots of maize. Lower concentration of oil field wastewater did not altered total number of maize leaves and secondary roots (Fig. 4). Total number of leaves and secondary roots were 2 and 3.8 at 5% while 1 and 2.7 at 10% oil field wastewater concentrations respectively (Fig. 4).

Effect of oil field wastewater pollution on photosynthetic pigments of maize leaves: Our results showed a decreased pattern in photosynthetic pigments contents of maize leaves treated with oil field waste water treatments. Chlorophyll a and b contents were concentration dependent and decreased by 5 and 10% compared to 1% oil field wastewater concentration. Chlorophyll “a” contents were 3.62, 3.2, 2.37, chlorophyll “b” were 2.24, 1.44, 0.67 and carotenoids were 3, 2 and 1 mg/g FW when maize leaves treated with 1, 5 and 10% oil field wastewater respectively (Fig. 5). Maximum inhibition of chlorophyll “a” and “b” were observed at 10% oil field wastewater concentration.

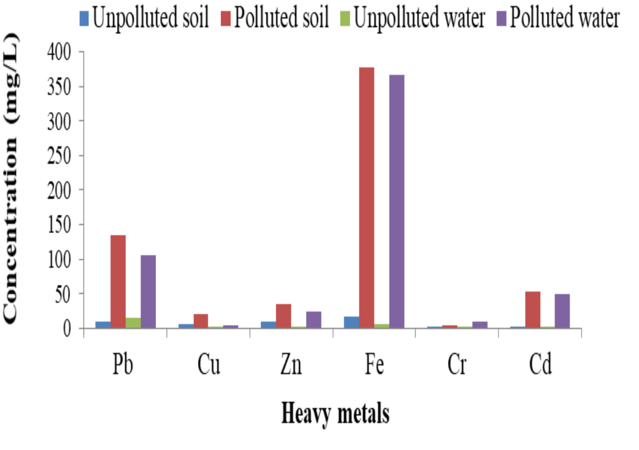

Fig .1. Concentrations of different heavy metals in oil field polluted and unpolluted soil and water.

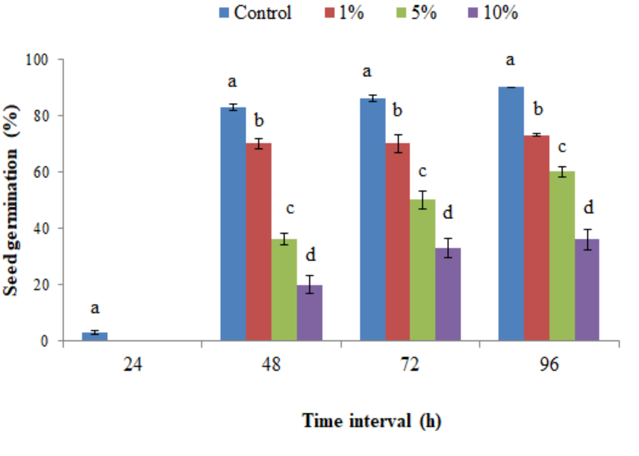

Fig. 2. Effect of oil field waste water on seed germination of maize at 24, 48, 72 and 96 h.

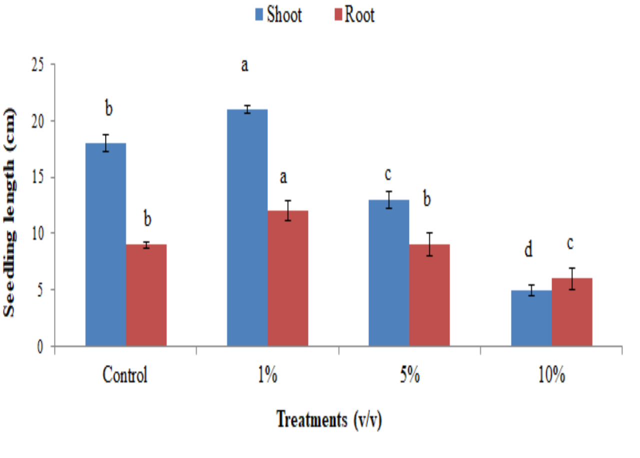

Fig. 3 A. Effect of oil field waste water on maize shoot/root length after 10 days.

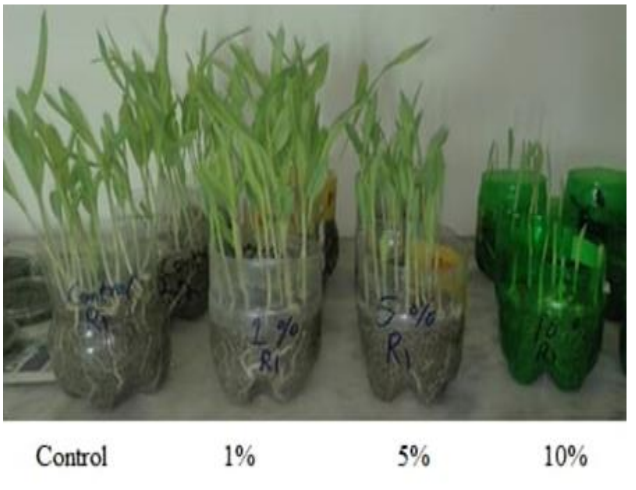

Fig. 3 B. Maize seedlings treated with 1, 5 and 10% concentrations of oil field waste water.

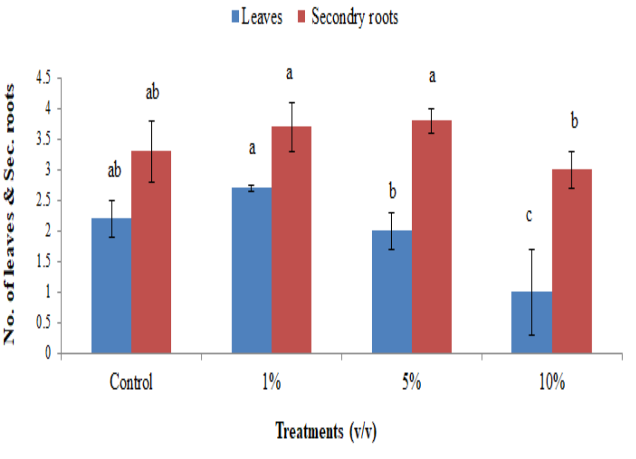

Fig. 4. Effect of oil field waste water on number of leaves and secondary roots of maize after 10 days.

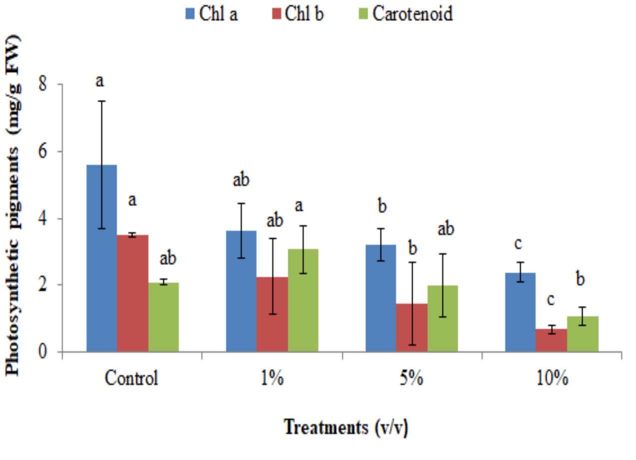

Fig. 5. Effect of oil field waste water on chlorophyll "*a*", "*b*" and carotenoids content in maize leaves after 10 days.

Effects of oil field wastewater on ionic (Na^+, Ca^{2+} and K^+) concentration in maize seedlings: Different ions play vital role in plant seedlings. Present results revealed that Na^+ and Ca^{2+} ions decreases while K^+ ion increases in maize shoot treated with 5 and 10% oil field waste water concentrations (Fig. 6A, B, C). On the other hand, concentrations of Na^+ and Ca^{2+} ions increases, while K^+ concentration decreases in maize roots treated with oil field waste water concentrations. In shoots, Na^+, Ca^{2+} and K^+ contents were 11.2, 9.03 and 54.1 mg/g DW at 10% oil field wastewater concentrations. In roots, Na^+, Ca^{2+} and K^+ contents were 31.53, 33.7 and 9.4 mg/g DW at 10% oil field wastewater concentrations (Fig. 6A, B, C).

Effects of oil field wastewater on total soluble sugar in maize seedlings: Results indicated that 5 and 10% concentration of oil field waste water significantly decreased total soluble sugar contents in maize seedlings as compared to control. Total soluble sugar were observed 8, 2.41 and 1.37 mg/g DW at 1, 5 and 10% oil field wastewater concentrations in maize shoot (Fig. 7). It was noted that 10% oil field wastewater concentration significantly decreased total soluble sugar in maize shoot compared to 1 and 5% concentrations. Total soluble sugar contents were 16.24, 14.87 and 5.45 mg/g DW in maize roots treated with 1, 5 and 10% oil field waste water concentrations (Fig. 7).

Effects of oil field wastewater pollution on antioxidant enzymes activities in maize: The effects of different concentrations of oil field wastewater were examined on various antioxidant enzymes in maize seedlings describe below; Total SOD contents improved in shoots and roots of maize with the increase of oil field waste water concentrations. The highest SOD contents were observed at 10% oil field wastewater concentration which was 2.41 µmole/mg in shoot and 1.93 µmole/mg in maize root (Fig. 8A). Similarly, POD contents gradually increased with the increase of oil field waste water concentrations both in maize shoot and root. The observed POD contents were 1.66, 3.53 and 4.43 µmole/mg in maize shoot treated with 1, 5, 10% oil field wastewater concentrations (Fig. 8B). The higher POD content was 5.6 µmole/mg in maize roots treated with 10% oil field wastewater concentration. Oil field wastewater were also applied on maize seedling to determine activity of catalase enzyme. Maize shoot and root treated with 10% oil field wastewater have 34.13 and 28.84 µmole/mg catalase (CAT) contents respectively (Fig. 8C). Activity of ascorbate peroxidase (APX) enzyme was also altered by oil field wastewater concentrations. Maximum APX enzyme activity were 0.17 and 0.20 µmole/mg in maize shoot and root treated with 10% oil field wastewater concentrations, respectively (Fig. 8D).

Discussion

Oil field wastewater pollution contaminates the soil with heavy metals (Gabbasova & Suleymanov, 2010) that disturb various morphological and biochemical processes in growing plants (Vwioko *et al.*, 2006). The current results are also in line with the previous finding mentioning that heavy metals increase in soil if oil field waste water is present (Fig. 1). Accumulation of heavy metals occurred in plants growing near industrial zone which releases huge amount of toxic materials (Shahnaz *et al*., 2021). Seed germination and seedling are important growth characters for the survival of any plant/crop in the environment. Results of the present research work indicated that maize seed germination did not alter by 1% but significantly decreased by 5 and 10% oil field wastewater concentrations (Fig. 2). Previous studies revealed that seed germination of guinea corn was affected by different concentrations of oil field waste water pollution (Lale *et al*., 2014). Olubodun & Eriyamremu (2013) reported that germination of maize seeds were significantly decreased by higher concentrations of oil field waste water. It is possible that the negative effects are due to toxic compounds or heavy metals present in oil field wastewater.

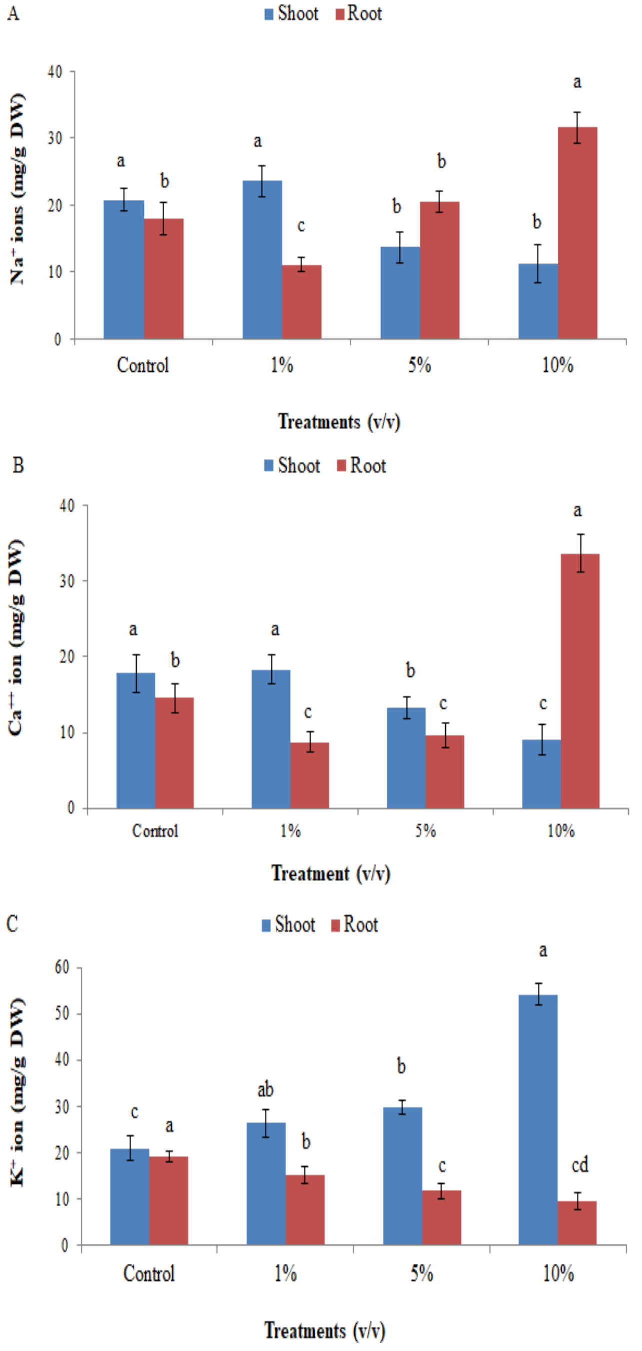

Fig. 6. Effects of oil field waste water on Na^+ (A), Ca^{++} (B) and K^+ (C) ions contents in maize shoot/root after 10 days.

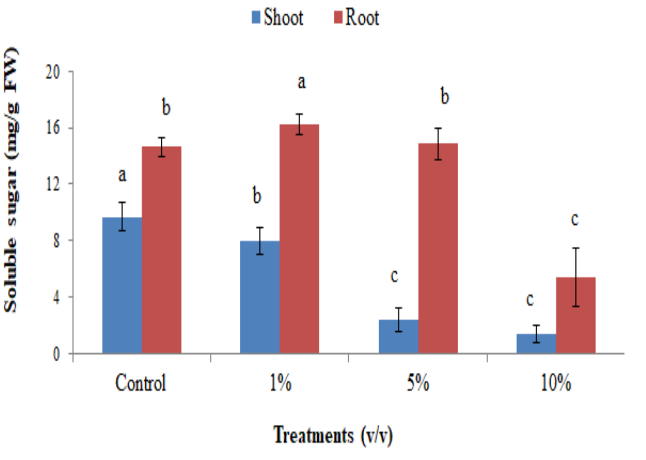

Fig. 7. Effect of oil field waste water on soluble sugar content of maize seedlings after 10 days.

Oil field wastewater concentrations also significantly reduced seedling growth of different crops/plants. This is due to the presence of different heavy metals in higher concentrations in oil field waste water (Firiappah *et al*., 2014; Ogbuehi *et al*., 2014). Present results revealed that maize seedling growth was significantly decreased by 5 and 10% oil field waste water concentrations (Fig. 3A, B). Oil field waste water decreased growth by limiting various nutrient availability to plant thus leading to compromised growth rate as was depicted in root length of onion (Odeigah *et al*., 1997). Generally, the overall morphology of plant is highly dependent on root efficiency for nutrient and water absorption. The soil affected by oil field wastewater imposes various stresses on plant which retarded root penetration in soil and thus leading to weak growth of roots. These weak roots are unable to take up nutrients more efficiently from soil and result in decreased seedling growth (Lale *et al*., 2014). Roots and secondary roots are highly susceptible for any source of contamination which more adversely affect the morphology and growth probably due to its direct contact with oil field waste water (Firiappah *et al*., 2014).

Photosynthetic pigments are the key pigments for plant growth because it is mandatory for the synthesis of glucose. Present results showed that 10% concentrations of oil field wastewater decreased chlorophyll a, b and carotenoids contents in maize leaves (Fig. 5). This decrease in photosynthetic pigments may be due to hindrance created by oil field wastewater in the biosynthesis of photosynthetic pigments (Ibemesim, 2010). Oil field waste water application degrading essential metabolites in plants leaves and causes chlorosis (Ibemesim, 2010). In many plants, oil field waste water inhibited biosynthesis of photosynthetic pigments and photosynthetic process (Akujobi *et al*., 2011; Al-Hawas *et al*., 2012; Baruah, 2014).

Literature revealed that oil field waste water significantly altered the presence of ionic contents like sodium (Na^+) calcium (Ca^{+2}) and potassium (K^+) in plants (Uhegbu *et al*., 2012). The present study results also showed that 5 and 10% concentrations of oil field wastewater significantly decreased Na^+ and Ca^{+2} ions while increased K^+ ion in maize shoot (Fig. 6A, B, C). Application of oil field waste water extensively altered

Na^{+}, Ca^{+2} and K^{+} ions concentrations in plants (Shukry *et al*., 2013). Potassium (K^{+}) ion has a critical role in the cell membrane permeability and regulation of stomata. Potassium ions are taken by plant as a cation to compensate anions (Marschner, 1995). Increase in Ca^{++} ions contents have been related with membrane stability and safety of plants against the negative impacts of hydrogen ions, salt contents and different poisonous ions found in polluted environment (Taiz & Zeiger, 2004). Therefore, it is possible that lowering Ca^{++} and Na^{+} ions contents might lead to the degradation of membrane and susceptibility of the plants to different stresses.

Total soluble sugar is a key indicator for the physiological growth in plants. Results confirmed that soluble sugar contents notably reduced at 5 and 10% treatments of oil field waste water in maize seedlings (Fig. 7). Sugar contents activated different photosynthetic and secondary metabolism related genes in plants (Chaves *et al*., 2002). Oil field wastewater reduced soluble sugar in sedge plant leaves thus reducing photosynthesis process (Korovetska *et al*., 2009). Taken together the previous and present results, it is observed that oil field waste water significantly decreased soluble sugar in maize seedlings.

Inside plants, naturally some specific antioxidant enzymes are produced to adjust the amount of reactive oxygen species (ROS) (Gratalo *et al*., 2005). Higher concentration of oil field waste water increased activities of SOD, POD, CAT and APX enzymes in maize seedlings (Fig. 8A, B, C, D). Treatments of oil field wastewater increased heavy metals which synthesized ROS caused oxidative damage and peroxidation of lipids (Malook *et al*., 2017; Maurya, 2020). Plants produced antioxidant enzymes to minimize the negative effects of ROS produced during stresses (Maurya, 2020). Super oxide dismutase (SOD) performs an essential function by converting super oxide radicals to hydrogen peroxide and oxygen (Tanyolac *et al*., 2007). Presence of increased SOD activity in oil field wastewater treated plants may be due to the production of lipid peroxidation (Tanyolac *et al*., 2007; Olubudon & Eriyamremu, 2015).

Catalase (CAT) is an essential enzyme that converts hydrogen peroxide to water and molecular oxygen (Mittler, 2002). ROS normally damaged the plant cells but on the other hand, CAT enzyme reduced the poisonous effects of ROS by converting into nontoxic form (Edema, 2010). Increase CAT contents during application of oil field wastewater in maize seedling might be the indication of reduction of stress in plant cells. So, any hydrogen peroxide molecule produced during stress eliminated by CAT enzyme (Edema, 2010). Oil field wastewater application also increased APX activity in maize seedlings. Odjegba & Badejo (2013) reported the increase of APX in *Capsicum annum* due to oil field wastewater treatments. APX is useful to convert hydrogen peroxide into water using ascorbate as a substrate (Odjegba & Badejo, 2013). Therefore, the previous and present results indicated that the increased production of different antioxidant enzymes in maize seedling reduced the abiotic stress produced due to oil field waste water pollution.

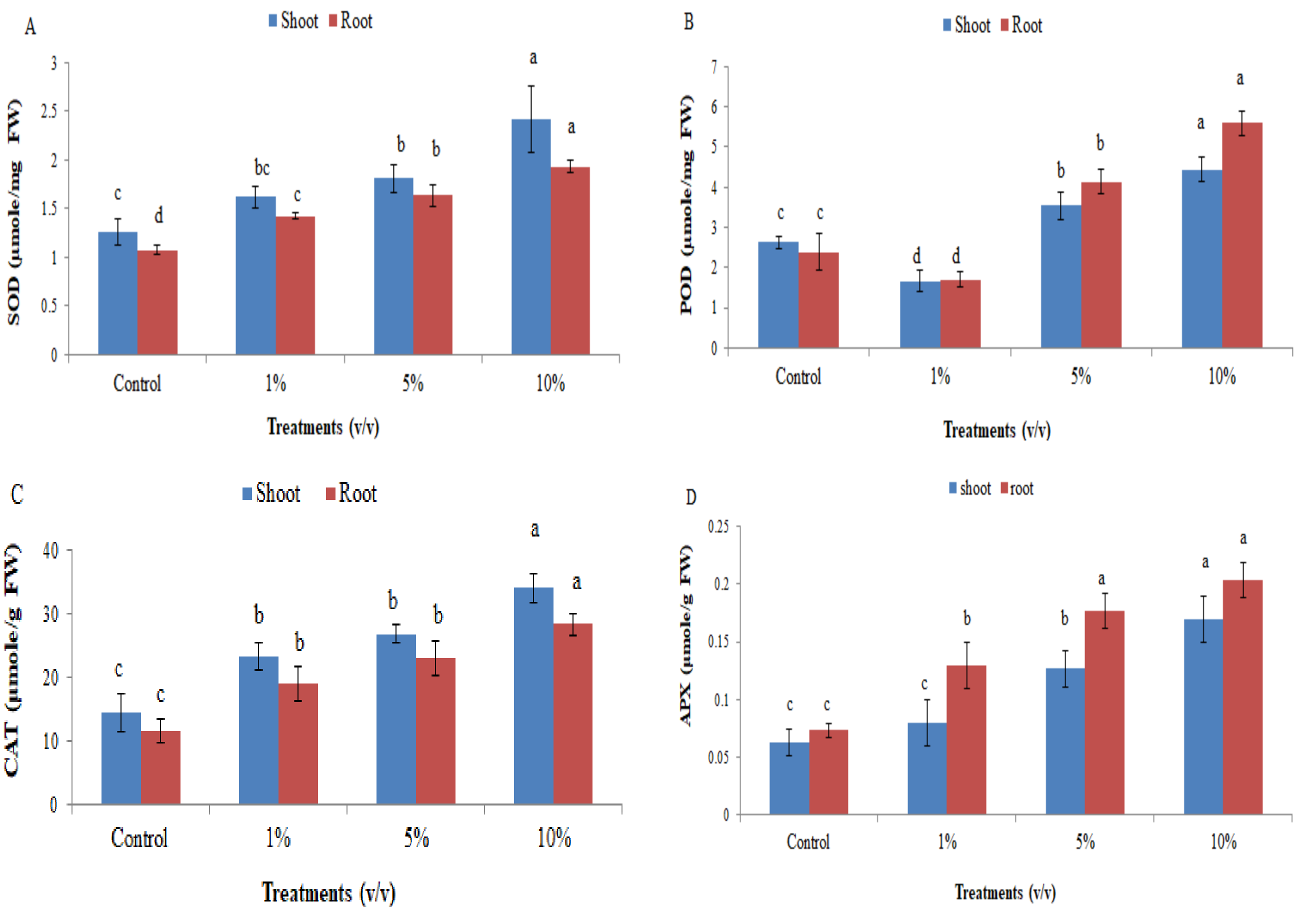

Fig. 8. Effects of oil field wastewater concentrations on SOD (A), POD (B), CAT (C) and APX (D) activities in maize seedlings after 10 days.

Conclusion

Oil field wastewater concentrations were applied on maize seeds to examine the morphological, physiological and antioxidant enzymatic responses. Results indicated that oil field wastewater concentrations significantly reduced morphological and physiological growth parameters of maize. It was found that activities of antioxidant enzymes increased in maize seedling treated with different concentrations of oil field wastewater. These results suggested that maize seedlings develop an alternate mechanism including the activation of antioxidant enzymes especially POD, SOD, CAT and APX to minimize the negative effects of oil field wastewater concentrations. The activation of antioxidants enzymes might be protective response in maize seedling against the stress created by oil field wastewater treatments.

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